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(54) Title: GLUCAGON-LIKE PEPTIDE AND INSULINOTROPIN DERIVATIVES (57) Abstract This invention relates to derivatives of glucagon-like peptide 1 (GLP-1), truncated GLP-1, insulinotropin and truncated insulinotropin which have a pI of about 4.0 or less or a pI of about 7.0 or greater. The derivatives of GLP-1, truncated GLP-1, insulinotropin and truncated insulinotropin within the scope of this invention are particularly suited for delivery to a mammal by iontophoresis. This invention also relates to methods for enhancing insulin action in a mammal with said derivatives and to pharmaceutical compositions comprising said derivatives. Further still, this invention relates to new uses of certain known derivatives of insulinotropin and truncated insulinotropin to enhance insulin action in a mammal by iontophoretic administration of such derivatives.		

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GLUCAGON-LIKE PEPTIDE AND INSULINOTROPIN DERIVATIVESField of the Invention

This invention relates to derivatives of glucagon-like peptide 1 (GLP-1), truncated GLP-1, insulinotropin and truncated insulinotropin. More specifically, this invention relates to derivatives of GLP-1, truncated GLP-1, insulinotropin and truncated insulinotropin, and the pharmaceutically acceptable salts thereof, which have a pI of about 4.0 or less or a pI of about 7.0 or greater. The derivatives of GLP-1, truncated GLP-1, insulinotropin and truncated insulinotropin within the scope of this invention are particularly suited for delivery to a mammal by iontophoresis. The derivatives of this invention have insulinotropic activity and are useful for enhancing insulin action in a mammal. The methods of treatment of this invention comprise administering to a mammal an effective amount of a derivative of GLP-1, truncated GLP-1, insulinotropin or truncated insulinotropin. Further, this invention relates to pharmaceutical compositions comprising said derivatives of GLP-1, truncated GLP-1, insulinotropin and truncated insulinotropin. Further still, this invention relates to new uses of certain known derivatives of insulinotropin and truncated insulinotropin to enhance insulin action in a mammal by iontophoretic administration of such derivatives.

Background Art

The amino acid sequence of GLP-1 is known as
His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 1).

GLP-1 is disclosed by Lopez, L. C., *et al.*, P.N.A.S., USA 80:5485-5489 (1983); Bell, G. I., *et al.*, Nature 302:716-718 (1983); Heinrich, G., *et al.*, Endocrinol. 115:2176-2181 (1984) and Ghiglione, M., *et al.*, Diabetologia 27:599-600 (1984).

GLP-1 is known to be naturally processed through conversion to a 31-amino acid peptide having amino acids 7-37 of GLP-1 (7-37). This processing reportedly occurs in the pancreas and intestine. The 7-37 peptide, herein referred to alternatively as insulinotropin, is a hormone that has insulinotropic activity.

Insulinotropin has the following amino acid sequence:
His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2).

Insulinotropin, certain derivatives thereof and the use thereof to treat Diabetes mellitus in a mammal are disclosed in PCT/US87/01005 (WO87/06941), published

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November 19, 1987. The teachings thereof are incorporated herein by reference. Derivatives of insulintropin disclosed in PCT/US87/01005 include polypeptides which contain or lack one or more amino acids that may not be present in the naturally-occurring sequence. Further derivatives of insulintropin disclosed in PCT/US87/01005

5 include certain C-terminal salts, esters and amides where the salts and esters are defined as OM where M is a pharmaceutically acceptable cation or a lower (C₁-C₆) branched or unbranched alkyl group and the amides are defined as -NR²R³ where R² and R³ are the same or different and are selected from the group consisting of hydrogen and a lower (C₁-C₆) branched or unbranched alkyl group.

10 Certain other polypeptides, herein alternatively referred to as truncated insulintropin, having insulintropic activity and the derivatives thereof are disclosed in PCT/US89/01121 (WO 90/11296). Those polypeptides, referred to therein as GLP-1 (7-36), GLP-1 (7-35) and GLP-1 (7-34) have the following amino acid sequences, respectively.

15 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3);
His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly (SEQUENCE ID NO: 4); and
His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5).

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Derivatives of the polypeptides disclosed in PCT/US89/01121 include polypeptides having inconsequential amino acid substitutions, or additional amino acids to enhance coupling to carrier protein or to enhance the insulintropic effect thereof. Further derivatives of insulintropin disclosed in PCT/US89/01121 include certain C-

25 terminal salts, esters and amides where the salts and esters are defined as OM where M is a pharmaceutically acceptable cation or a lower branched or unbranched alkyl group and the amides are defined as -NR²R³ where R² and R³ are the same or different and are selected from the group consisting of hydrogen and a lower branched or unbranched alkyl group.

30 Delivery of therapeutically effective polypeptides to a mammal present certain problems well known to those skilled in the art. Oral administration of a polypeptide, without some form of delivery device, will generally be unsuccessful due to the low inherent permeability of the intestine and other processes such as chemical

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degradation in the stomach and intestine. Transdermal administration of polypeptides affords the possibility of providing therapeutically effective polypeptides to a mammal without subjecting the polypeptides to degradation in the gut. Various approaches for transdermal administration of pharmaceutically active compounds are known to those skilled in the art. One such approach for transdermal administration is a method known to those skilled in the art as iontophoresis.

Iontophoresis involves the application of a potential electrical gradient across the skin in conjunction with the surface co-application of therapeutic agents. To accomplish this, two electrodes are required in addition to a drug reservoir and a power source. Various type of iontophoretic devices are described by Tyle, P., *Pharmaceutical Research* 3:318-326 (1986). An example of an electrode for use in iontophoresis is disclosed in U.S. 4,950,229, the teachings of which are incorporated herein by reference. The result of iontophoresis is transport of therapeutic agents across the skin to either local or systemic sites. Further, iontophoresis is known to comprise the application of different voltage patterns including such methods as electroporation or pulsed current techniques.

A low level of electrical current has been used to transdermally administer leuprolide, a synthetic 9 amino acid leutinizing hormone releasing hormone analog. Meyer, B. R., et al., *Clin. Pharmacol. Ther.* 44:607-612 (1988). A study on the iontophoretic delivery of insulin to rats has been reported. Siddiqui, O., et al. *J. Pharmaceutical Sciences* 76:341-345 (1987). Studies on transdermal iontophoresis of gonadotropin releasing hormone and thyrotropin releasing hormone have been reported. Miller, L. L., et al., *J. Pharmaceutical Sciences* 79:490-493 (1990) and Burnette, R. R., et al., *J. Pharmaceutical Sciences* 75:738-743 (1986). Ethanol, has been disclosed as enhancing iontophoretic transdermal delivery of leuprolide and CCK-8 (cholecystokinin-8) analog. Srinivasan, V. et al., *J. Pharmaceutical Sciences* 79:588-591 (1990).

Disclosure of the Invention

This invention relates to polypeptide derivatives of glucagon-like peptide 1 (GLP-1) and truncated GLP-1 comprising the primary structure



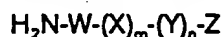
wherein W is an amino acid sequence selected from the group consisting of

His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 1) and

5 His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 6);

and the pharmaceutically-acceptable salts thereof wherein the derivative has a pI of about 4.0 or less, or a pI of about 7.0 or greater and when processed in a mammal results in a polypeptide derivative having insulinotropic activity.

10 This invention further relates to polypeptide derivatives of GLP-1 and truncated GLP-1 described above comprising the primary structure



and the pharmaceutically-acceptable salts thereof wherein W is as defined above; m is zero or one; n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO_2R^1 or CONR^2R^3 wherein R^1 is H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when m is one, n is zero and X is a basic L-amino acid residue, or m is zero, n is one and Y is a basic L-amino acid residue, or m and n are both one and one or both of X and Y are a basic L-amino acid residue; R^1 is $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when m and n are both zero, or m is one, n is zero and X is a neutral L-amino acid residue, or m is zero, n is one and Y is a neutral L-amino acid residue, or m and n are both one and both X and Y are a neutral L-amino acid residue; and R^2 and R^3 are each, independently, H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl.

25 Further still, this invention relates to derivatives of polypeptides comprising the primary structure



wherein R is an amino acid sequence selected from the group consisting of

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2),
 30 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3),
 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly (SEQUENCE ID NO: 4) and

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His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5);

- and the pharmaceutically-acceptable salts thereof wherein the derivative has a pI of about 4.0 or less, or a pI of about 7.0 or greater and having insulinotropic activity, provided that said derivative is not a C-terminal (C₁-C₆)straight or branched-chain alkyl ester and provided further that said derivative is not a C-terminal carboxamide of the formula CONR²R³ wherein R² and R³ are each, independently, H or (C₁-C₆)straight or branched-chain alkyl.

- This invention also relates to derivatives of the polypeptides described immediately above comprising the primary structure



- and the pharmaceutically-acceptable salts thereof wherein R is as described above; n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO₂R¹ or CONR²R³ wherein R¹ is H or (C₁-C₆) straight or branched-chain alkyl when n is zero and X is a basic L-amino acid residue, or n is one and one or both of X and Y are a basic L-amino acid residue; R¹ is (C₁-C₆)straight or branched-chain alkyl when n is zero and X is a neutral L-amino acid residue, or n is one and both X and Y are a neutral L-amino acid residue; and R² and R³ are each, independently, H or (C₁-C₆)straight or branched-chain alkyl.

- Preferred derivatives of this invention are those having a pI of about 8.5 or greater. Still other preferred derivatives of such polypeptides are those wherein R is His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2). Even more preferred are such derivatives wherein R is

- His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2);

n is zero and X is Arg or n is one and X and Y are each Arg. Still more preferred derivatives are such preferred derivatives wherein Z is CO₂R¹ and R¹ is H or Z is CONR²R³ and R² and R³ are each H.

- This invention further relates to methods of enhancing insulin action in a mammal which methods comprise administering to said mammal an effective amount of a derivative according to this invention. A preferred method of enhancing insulin action according to this invention comprises treatment of Type II diabetes. A preferred

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method of administration of such derivatives is iontophoretic transdermal administration.

Still further yet, this invention relates to a method of enhancing insulin action in a mammal which comprises iontophoretically administering to said mammal an effective amount of a derivative of a polypeptide comprising the primary structure



wherein R is an amino acid sequence selected from the group consisting of

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2),

10 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3),

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly (SEQUENCE ID NO: 4) and

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5);

15 and the pharmaceutically-acceptable salts thereof wherein m is zero or one; n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO_2R^1 or CONR^2R^3 wherein R^1 is H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when m is one, n is zero and X is a basic L-amino acid residue, or m is zero, n is one and Y is a basic L-amino acid residue, or m and n are both one and one or both of X and Y are a basic L-amino acid residue; R^1 is $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when m and n are both zero, or m is one, n is zero and X is a neutral L-amino acid residue, or m is zero, n is one and Y is a neutral L-amino acid residue or m and n are both one and both X and Y are a neutral L-amino acid residue; and R^2 and R^3 are each, independently, H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl.

25 The derivatives of this invention can also be used in connection with other therapies such as other anti-diabetic agents (e.g., sulfonylureas).

A preferred method of iontophoretically administering a derivative of a polypeptide as described immediately above comprises iontophoretically administering said derivative wherein R is

30 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2);

m is zero and n is zero,

with a still more preferred method comprising said preferred method wherein Z is

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CONR²R³ and R² and R³ are each hydrogen.

This invention further relates to pharmaceutical compositions comprising derivatives of polypeptides according to this invention. Such pharmaceutical compositions are useful in enhancing insulin action in a mammal. Thus, the pharmaceutical compositions of this invention are particularly suited for treatment of certain diabetic conditions such as Type II diabetes.

Detailed Description

The term "derivative", as used throughout this Specification and the appendant claims, includes, but is not limited to, polypeptides comprising the primary structure shown, wherein one or more L-amino acids are included at the C-terminus thereof; wherein the C-terminal carboxyl group forms an ester with a (C₁-C₆) straight or branched-chain alkyl group; wherein the C-terminal carboxyl group forms a carboxamide or substituted carboxamide; wherein the acidic amino acid residues (Asp and/or Glu) form an ester or carboxamide; and combinations thereof.

The term "basic L-amino acid residue", as used throughout the Specification and the appendant claims, includes, but is not limited to, the common amino acids Lys, Arg and His.

The term "neutral L-amino acid residue", as used throughout this Specification and the appendant claims, includes, but is not limited to, Ala, Val, Leu, Ile, Pro, Met, Phe, Trp, Gly, Ser, Thr, Cys, Tyr, Asn and Glu. While Gly is not, technically, an L-amino acid residue due to the presence of only hydrogen at the α -carbon in addition to the carboxyl and amino groups, it is referred to herein as an L-amino acid for the sake of simplicity.

The foregoing classification of L-amino acid residues as basic or neutral is based upon the net charge of the corresponding amino acid at pH 7.0.

As used throughout this Specification and the appendant claims, the "term pI" refers to the theoretical pI which is calculated using commercial software known as PCGENE (IntelliGenetics, Inc., 700 East El Camino Real, Mountain View, CA 94040).

Included within the scope of this invention are polypeptides having homology to the polypeptides described above, which homology is sufficient to impart insulinotropic activity to such polypeptides. Also included within the scope of this invention are variants of the polypeptides described above, which variants comprise inconsequential amino acid substitutions and have insulinotropic activity.

The phrase "enhancing insulin action", as used throughout this Specification and the appendant claims, includes, but is not limited to, one or more of increasing insulin synthesis, increasing insulin secretion, increasing glucose uptake by muscle and fat and decreasing glucose production by the liver.

5 The polypeptides of this invention are prepared by various methods well known to those skilled in the art. For example, the polypeptides can be synthesized using automated peptide synthesizers such as an Applied Biosystems (ABI) 430A solid phase peptide synthesizer. Alternatively, the polypeptides wherein Z is CO₂H of this invention
10 can be prepared using recombinant DNA technology wherein a DNA sequence coding for the polypeptide is operably linked to an expression vector and used to transform an appropriate host cell. The transformed host cell is then cultured under conditions whereby the polypeptide will be expressed. The polypeptide is then recovered from the culture. Further still, a combination of synthesis and recombinant DNA techniques can
15 be employed to produce the amide and ester derivatives of this invention and/or to produce fragments of the desired polypeptide which are then joined by methods well known to those skilled in the art.

Derivatives of the polypeptides according to this invention are prepared by methods well known to those skilled in the art. For example, C-terminal alkyl ester
20 derivatives of the polypeptides of this invention are prepared by reacting the desired (C₁-C₆)alkanol with the desired polypeptide in the presence of a catalytic acid such as HCL. Appropriate reaction conditions for such alkyl ester formation include a reaction temperature of about 50°C and reaction times of about 1 hour to about 3 hours. Similarly, derivatives of the polypeptides of this invention comprising (C₁-C₆)alkyl esters
of the Asp and/or Glu residues within the polypeptide can be so formed.

25 Preparation of carboxamide derivatives of the polypeptides of this invention are also prepared by solid phase peptide synthesis methods well known to those skilled in the art. For example, see, Solid Phase Peptide Synthesis, Stewart, J. M. et al., Pierce Chem. Co. Press, 1984. When derivatives of the polypeptides according to this invention having a pI of about 4.0 or less are desired, such derivatives can be prepared
30 by various methods well known to those skilled in the art. For example, deamidation of a glutamine residue to produce a glutamic acid residue, alkylation or amidation of free amino groups at the N-terminus and/or at the epsilon amino groups of lysine residues, or a combination thereof will result in derivatives having lower pI values. To

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decreases the pI of a derivative of insulinotropin to less than about 3.89, modification of any two of the available amino groups, or modification of at least one amino group and deamidation of the glutamine are necessary. Deamidation of glutamine residues is readily accomplished by suspension of the desired polypeptide of this invention in
5 water at a pH greater than 8 for a period of several hours.

For example, depending on the pH of the reaction, acetylation of insulinotropin under basic conditions can afford the derivative wherein both amidation of the N-terminal amino group and both of the epsilon amino groups has occurred. The result is a derivative with a theoretical pI of 3.61. Alternatively, N-terminal acetylation
10 combined with deamidation of the single glutamine residue of insulinotropin to a glutamic acid residue yields a derivative with a theoretical pI of 4.11.

As an alternative method for reducing the pI, a cyclic anhydride can be reacted with a polypeptide to block a basic residue and introduce an acidic residue. By way of example and not of limitation, a solution of insulinotropin (SEQUENCE ID NO.: 2)
15 in DMF in the presence of 8 equivalents of triethylamine and 8 equivalents of succinic anhydride affords the N-succinate derivative of insulinotropin at the N-terminus and the Lys₂₀ and Lys₂₈ residues thereof.

Alternatively, or in combination with the above, derivatives of the polypeptides of this invention can be prepared by modifying the DNA coding sequence for such
20 polypeptide so that a basic amino acid residue is replaced with an acidic or neutral amino acid residue, or a neutral amino acid residue is replaced with an acidic amino acid residue. Such changes in polypeptide primary sequence can also be accomplished by direct synthesis of the derivative. Such methods are well known to those skilled in the art. Of course, such derivatives, to be useful in the practice of this
25 invention, must achieve an insulinotropic effect.

The insulinotropic activity of a polypeptide derivative according to this invention, wherein said polypeptide does not include amino acids 1-6 of GLP-1 or truncated GLP-1, is determined as follows.

Pancreatic islets are isolated from pancreatic tissue from normal rats by a
30 modification of the method of Lacy, P. E., et al., Diabetes, 16:35-39 (1967) in which the collagenase digest of pancreatic tissue is separated on a Ficoll gradient (27%, 23%, 20.5% and 11% in Hanks' balanced salt solution, pH 7.4). The islets are collected from the 20.5%/11% interface, washed and handpicked free of exocrine and other tissue

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under a stereomicroscope. The islets are incubated overnight in RPMI 1640 medium supplemented with 10% fetal bovine serum and containing 11 mM glucose at 37°C and 95% air/5% CO₂. The islets are then transferred to RPMI 1640 medium supplemented with 10% fetal bovine serum and containing 5.6 mM glucose. The islets are incubated
5 for 60 minutes at 37°C, 95% air/5% CO₂. The polypeptide derivative to be studied is prepared at 1 nM and 10 nM concentrations in RPMI medium containing 10% fetal bovine serum and 16.7 mM glucose. About 8 to 10 isolated islets are then transferred by pipette to a total volume of 250 µl of the polypeptide derivative containing medium in 96 well microtiter dishes. The islets are incubated in the presence of the polypeptide
10 derivative at 37°C, 95% air/5% CO₂ for 90 minutes. Then, aliquots of islet-free medium are collected and 100 µl thereof are assayed for the amount of insulin present by radioimmunoassay using an Equate Insulin RIA Kit (Binx, Inc., Portland, ME).

Pharmaceutical compositions comprising polypeptide derivatives according to this invention can be prepared according to methods well known to those skilled in the
15 art. For example, the polypeptide derivatives can be combined with a pharmaceutically acceptable diluent or carrier. When the polypeptide derivatives of this invention are to be administered intravenously, intramuscularly or subcutaneously, appropriate sterile diluent is employed as is well known in the art. Such pharmaceutical compositions will comprise a sufficient amount of the polypeptide derivative so that an appropriate
20 dosage, as hereinbelow described, can be administered over an appropriate period of time.

For iontophoretic delivery of a polypeptide derivative according to this invention, various compositions can be prepared. The polypeptide derivative can be included in a solution or as part of a gel or foam. It is preferable, however, that the polypeptide
25 derivative in such composition have the same or approximately the same charge as the electrode in the drug reservoir of the iontophoretic device to be employed. The charge of the derivative can be controlled, for example, by the use of an appropriate buffer. When using a buffer, it is preferable to employ a buffer which has a charge opposite to that of the particular polypeptide derivative to be administered. Alternatively, the
30 polypeptide derivative may act as its own "buffer" if the appropriate salt form thereof is employed. Variables in such compositions include the concentration of the polypeptide derivative, the buffer concentration when present, the ionic strength of the composition and the nonaqueous cosolvents. In general, to achieve the highest transport efficiency

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by iontophoresis with such compositions, it is preferable to minimize the concentration of all ionic species except the polypeptide derivative in such compositions. Adjustment of such concentrations are within the skill of those who practice in the art, enabled by the disclosure herein.

5 A variety of iontophoretic devices are known. Various electrode materials for use with such devices are known and available. Such electrodes include those made of platinum or silver-silver chloride. The differences among electrodes are known to be associated with certain performance nuances. For example, use of platinum electrodes cause hydrolysis leading to the liberation of hydrogen ions and subsequent changes
10 in pH. Changes in pH, in turn, can influence the ionization state of the polypeptide derivative and, thus, the resulting iontophoretic transport thereof. Silver-silver chloride electrodes, on the other hand, do not hydrolyze water when used with iontophoretic devices. Such silver-silver chloride electrodes, however, require the presence of chloride ions which may compete for current-induced transport across the skin. Choice
15 of the appropriate electrode for use in iontophoretic administration of the polypeptide derivatives according to this invention is within the skill of those who practice in the art, enabled by the disclosure herein.

 In addition, the methods for evaluating iontophoretic delivery using a porcine skin flap, as described by Riviere, J. E., et al., J. Toxicol.-Cut. & Ocular Toxicol. 8:493-
20 504 (1989-1990) can be employed with the polypeptide derivatives and compositions of this invention.

 Dosages effective in treatment of adult onset diabetes will range from about 1 pg/kg to 1,000 μ g/kg per day when a polypeptide derivative of this invention is administered intravenously, intramuscularly or subcutaneously. A preferred dosage
25 range for intravenous infusion during and between meals is about 4 to 10 ng/kg/min or about 0.6 to 1.4 μ g/day based on a 100 kg patient. It is to be appreciated, however, that dosages outside of that range are possible and are also within the scope of this invention. The appropriate dosage can and will be determined by the prescribing physician and will be a result of the severity of the condition being treated as well as
30 the response achieved with the derivative being administered and the age, weight, sex and medical history of the patient. For iontophoretic administration of a polypeptide derivative according to this invention, a dosage range of about 500 to 1000 μ g/per day.

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Here, too, dosages outside of that range are possible and are within the scope of this invention.

EXAMPLE 1

$H_2N-R-X(Y)_n-Z$ wherein n is zero; X is arg; Z is $CONR^2R^3$ where R^2 and R^3 are H; and

- 5 R is His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2)

The title peptide was synthesized starting with a p-methylbenzhydrylamine (HCl salt) resin using an Applied Biosystems (ABI) 430A solid phase peptide synthesizer [mfr, address] using ABI Version 1.40 of the N-methylpyrrolidone/hydroxybenzotriazole

- 10 t-BOC cycles. The end cycle was selected to remove the ultimate t-BOC protecting group at the completion of the synthesis. The following amino acid side chain protection was used: Arg (Tos), Lys (Cl-Z), Trp (CHO), Glu (OCyHex), Tyr (Br-Z), Ser (Bzl), Thr (Bzl), Asp (OCyHex) and His (BOM). The synthesis cycles used were those provided by ABI with the following modifications: the delivery time of
15 hydroxybenzotriazole to the measuring loop was increased from 6 seconds to 10 seconds to assure reproducible and proper delivery thereof; and the delivery time of hydroxybenzotriazole to the activator vessel was increased from 12 seconds to 18 seconds. In order to prevent clogs in the instrument caused by vapors generated by the acetic anhydride reagent bottle, the reaction vessel cycle was modified to pressurize
20 the associated valve block after each delivery of acetic anhydride. The ABOC11 activator cycle was used for the activation of Glu in place of the normally used ABOC12. After final removal of the N-terminal t-BOC group, a total of 2.65 g of peptide-resin was obtained. Then, the formyl protecting group was removed from the Trp residue by treatment of the peptide-resin for 1 hour by gently shaking in a solution
25 containing 2 ml H_2O , 2 ml of 70% ethanolamine in methanol and 16 ml dimethylformamide. Then, the resin was filtered, washed successively with dimethylformamide (3 x 10 ml), methanol (3 x 10 ml) and dichloromethane (3 x 10 ml). The washed resin was dried in vacuo to yield 2.48 g of resin.

- To remove the peptide from the resin, 997 mg of the dried resin was treated with
30 liquid hydrogen fluoride/10% m-cresol at 0°C for 1 hour. Then, the hydrogenfluoride was removed by evaporation and the peptide was taken up in trifluoroacetic acid. The peptide was then precipitated using ethyl ether to yield 403 mg of peptide as a white solid. Analytical HPLC data suggested incomplete removal of the Trp formyl protecting

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group in the peptide. To remove the remaining formyl protecting groups, 40 mg of the peptide was dissolved in a mixture of 3.6 ml H₂O and 0.4 ml of 70% ethanolamine in methanol. The resulting solution was allowed to sit for 30 minutes at room temperature. Then, 0.35 ml of trifluoroacetic acid was added and the precipitate was collected by centrifugation (14,000 rpm, 5 min.). The collected precipitate was dissolved in 4 ml 6M guanidine-HCl and chromatographed by preparative reverse phase HPLC on a 1 inch VYDAC C18 column using a gradient system of 100% → 40%A, 0% → 60%B over a period of 60 min. at a flow rate of 10 ml/min. (A is 0.1% trifluoroacetic acid/95% H₂O/5% CH₃CN and B is CH₃CN) to afford 10.5 mg of the title peptide.

10 FAB MS: 3511.4 Da (parent + H predicted: 3511 Da)

EXAMPLE 2

H₂N-R-X-(Y)_n-Z wherein n is zero; X is arg; Z is CO₂R¹ where R¹ is H; and R is His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Glu-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2)

15 Using the procedure described in Example 1, modified to synthesize the title peptide, a total of 2.0 g of peptide resin was prepared. Treatment of 900 mg of the peptide resin with hydrogen fluoride according to the procedure of Example 1 yielded 340 mg of peptide after precipitation of the trifluoroacetic acid solution from ethyl ether. Then, as in Example 1, 40 mg of the peptide was treated in aqueous ethanolamine, acidified, precipitated and chromatographed to afford 10 mg of the title peptide.

20 FAB MS: 3511.9 Da (parent + H predicted: 3512 Da)

EXAMPLE 3

H₂N-R-X-(Y)_n-Z wherein n is one; X is arg; Y is arg; Z is CONR²R³ where R² and R³ are h; and R is His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-
 25 Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2)

Using the procedure described in Example 1, modified to synthesize the title peptide; a total of 1.3 g of peptide resin was prepared. Treatment of 1.3 g of the peptide resin with hydrogen fluoride according to the procedure of Example 1 yielded 671 mg of the peptide after precipitation of the trifluoroacetic acid solution from ethyl ether. Then, as in Example 1, 7 mg of the peptide was treated in aqueous ethanolamine, acidified, precipitate and chromatographed to afford 4.2 mg of the titl peptide.

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FAB MS: 3667.8 Da (parent + H predicted: 3667.81 Da)

EXAMPLE 4wherein n is one; X is arg; Y is arg; Z is CO_2R^1 where R^1 is H; and R is

5 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Ala-Ala-
 Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2)

Using the procedure described in Example 1, modified to synthesize the title peptide; a total of 2.66 g of peptide resin was prepared. Treatment of 1.1 g of the peptide resin with hydrogen fluoride according to the procedure of Example 1 yielded
 10 490 mg of the peptide after precipitation of the trifluoroacetic acid solution from ethyl ether. Then, as in Example 1, 10 mg of the peptide was treated in aqueous ethanolamine, acidified, precipitate and chromatographed to afford 3.8 mg of the title peptide.

FAB MS: 3669.1 Da (parent + H predicted: 3668.83 Da)

15

EXAMPLE 5

wherein n is zero; X is Arg; Z is CO_2R^1 wherein R^1 is H; and R is a derivative of His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2) where the derivative
 20 is N-terminal (N-alpha) succinoylated and the epsilon amine group of both

Lys residues are succinoylated

To a solution of 800 μ g (0.24 μ moles) of insulinotropin (SEQUENCE ID NO. 2) in 800 μ l of dimethylformamide (DMF) was added sequentially 200 μ g (2.0 μ moles) of succinic anhydride in 20 μ l of DMF and 200 μ g (2.0 μ moles) of triethylamine in 10 μ l of DMF. The clear solution was stirred for three hours at ambient temperature, affording a single major product by reverse phase HPLC analysis, according to the method of Example 1, in approximately 90% yield (based on HPLC peak areas). The product was added to 10 ml of water, acidified with trifluoroacetic acid to pH 2 and
 25 lyophilized to dryness. The dried lyophilizate was taken up in HPLC mobile phase and purified by preparative reverse phase HPLC, according to the method of Example 1, affording a homogeneous product shown by Plasma Desorption mass spectral analysis
 30

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to be tri-succinoyl substituted insulinotropin. Expected Mass (M + H): 3656.68 Da, Found: 3658.2 Da.

EXAMPLE 6



- 5 wherein n is zero; X is Arg; Z is CO_2R^1 where R^1 is H; and R is a derivative of His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2) where the derivative
is hexa- or hepta-succinoylated

- According to the method of Example 5, 100 μg (0.03 μmoles) instead of 800 μg
10 (0.24 μmoles) of insulinotropin (SEQUENCE ID NO.: 2) was reacted according to all of the other reactive amounts of Example 5 to afford a new product after stirring 16 hours at ambient temperature (as evidenced by HPLC analysis). The product, isolated according to the method of Example 5, was shown by ES-MS mass spectral analysis to be a mixture of hexa- and hepta-succinoylated insulinotropin. Expected Mass for
15 hexa-succinoylated insulinotropin: 3955.68 Da, Found: 3955.9 Da. Expected Mass for hepta-succinoylated insulinotropin: 4055.68 Da, Found: 4055.9 Da.

EXAMPLE 7



- wherein n is zero; X is Arg; Z is CO_2R^1 where R^1 is H; and R is
20 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2)

- One hundred micrograms (100 μg) of insulinotropin (SEQUENCE ID NO: 2) was dissolved in a solution of 50 μl of DMF containing 20 μl of aqueous tricine buffer (pH 8.75) and stirred at 37°C overnight. HPLC according to the method of Example 1
25 showed the presence of a new peak which was shown to co-elute with the product produced by total synthesis according to Example 2.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Andrews, Glenn C.
Daumy, Gaston O.
5 Francoeur, Michael L.
Larson, Eric R.
Pfizer Inc, (Non-US)
- (ii) TITLE OF INVENTION: GLUCAGON-LIKE PEPTIDE AND INSULINOTROPIN
DERIVATIVES
- 10 (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Gregg C. Benson, Pfizer Inc
(B) STREET: Eastern Point Road
(C) CITY: Groton
15 (D) STATE: CT
(E) COUNTRY: USA
(F) ZIP: 06340
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
20 (B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
25 (B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/899,073
(B) FILING DATE: 15-JUN-1992
- 30 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Benson, Gregg C.
(B) REGISTRATION NUMBER: 30,997
(C) REFERENCE/DOCKET NUMBER: PC8156AGCB
- (ix) TELECOMMUNICATION INFORMATION:
35 (A) TELEPHONE: (203) 441-4901
(B) TELEFAX: (203) 441-5221
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
40 (A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val
 1 5 10 15
 Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu
 5 20 25 30
 Val Lys Gly Arg Gly
 35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
 1 5 10 15
 Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 20 25 30

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
 1 5 10 15
 Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg
 20 25 30

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
 1 5 10 15
 Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly
 20 25

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 amino acids

(B) TYPE: amino acid

5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
 1 5 10 15
 10 Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys
 20 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 36 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

His Asp Glu Phe Glu Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val
 1 5 10 15
 20 Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu
 25 30
 25 Val Lys Gly Arg
 35

CLAIMS

1. A derivative of a polypeptide comprising the primary structure



wherein W is an amino acid sequence selected from the group consisting of

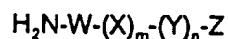
5 His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 1) and

His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-

10 Arg (SEQUENCE ID NO: 6);

and the pharmaceutically-acceptable salts thereof wherein the derivative has a pI of about 4.0 or less, or a pI of about 7.0 or greater and which derivative when processed in a mammal results in a polypeptide derivative having an insulintropic activity.

2. A derivative of a polypeptide according to claim 1 comprising the primary
15 structure



and the pharmaceutically-acceptable salts thereof wherein m is zero or one; n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO_2R^1 or CONR^2R^3 wherein R^1 is H or $(\text{C}_1\text{-C}_6)$ straight or
20 branched-chain alkyl when

- (a) m is one, n is zero and X is a basic L-amino acid residue, or
- (b) m is zero, n is one and Y is a basic L-amino acid residue, or
- (c) m and n are both one and one or both of X and Y are a basic L-amino acid residue;

25 R^1 is $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when

- (a) m and n are both zero, or
- (b) m is one, n is zero and X is a neutral L-amino acid residue, or
- (c) m is zero, n is one and Y is a neutral L-amino acid residue, or
- (d) m and n are both one and both X and Y are a neutral L-amino acid
30 residue;

and R^2 and R^3 are each, independently, H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl.

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3. A derivative of a polypeptide comprising the primary structure

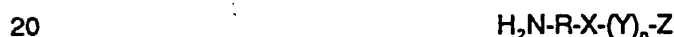


wherein R is an amino acid sequence selected from the group consisting of

- 5 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
 Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2),
 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
 Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3),
 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
 Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly (SEQUENCE ID NO: 4) and
 10 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
 Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5);

and the pharmaceutically-acceptable salts thereof wherein the derivative has a pI of about 4.0 or less, or a pI of about 7.0 or greater and having insulinotropic activity, provided that said derivative is not a C-terminal ($\text{C}_1\text{-C}_6$)straight or branched-chain alkyl ester and provided further that said derivative is not a C-terminal carboxamide of the
 15 formula CONR^2R^3 wherein R^2 and R^3 are each, independently, H or ($\text{C}_1\text{-C}_6$)straight or branched-chain alkyl.

4. A derivative of a polypeptide according to claim 3 comprising the primary structure



and the pharmaceutically-acceptable salts thereof wherein n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO_2R^1 or CONR^2R^3 wherein R^1 is H or ($\text{C}_1\text{-C}_6$) straight or branched-chain alkyl when

- (a) n is zero and X is a basic L-amino acid residue, or
 25 (b) n is one and one or both of X and Y are a basic L-amino acid residue;
 R^1 is ($\text{C}_1\text{-C}_6$)straight or branched-chain alkyl when

- (a) n is zero and X is a neutral L-amino acid residue, or
 (b) n is one and both X and Y are a neutral L-amino acid residue;

and R^2 and R^3 are each, independently, H or ($\text{C}_1\text{-C}_6$)straight or branched-chain alkyl.

30 5. A derivative of a polypeptide according to claim 4 and the pharmaceutically-acceptable salts thereof wherein R is

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
 Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2).

6. A derivative of a polypeptide according to claim 5 and the pharmaceutically-acceptable salts thereof wherein n is zero and X is Arg.

7. A derivative of a polypeptide according to claim 6 and the pharmaceutically-acceptable salts thereof wherein Z is CO_2R^1 and R^1 is H.

5 8. A derivative of a polypeptide according to claim 6 and the pharmaceutically-acceptable salts thereof wherein Z is CONR^2R^3 and R^2 and R^3 are each H.

9. A derivative of a polypeptide according to claim 5 and the pharmaceutically-acceptable salts thereof wherein n is one and X and Y are each Arg.

10 10. A derivative of a polypeptide according to claim 9 and the pharmaceutically-acceptable salts thereof wherein Z is CO_2R^1 and R^1 is H.

11. A derivative of a polypeptide according to claim 9 and the pharmaceutically-acceptable salts thereof wherein Z is CONR^2R^3 and R^2 and R^3 are each H.

15 12. A pharmaceutical composition comprising a derivative of a polypeptide according to claim 1.

13. A pharmaceutical composition comprising a derivative of a polypeptide according to claim 2.

20 14. A pharmaceutical composition comprising a derivative of a polypeptide according to claim 3.

15. A pharmaceutical composition comprising a derivative of a polypeptide according to claim 4.

25 16. A method of enhancing insulin action in a mammal which comprises administering to said mammal an effective amount of a derivative of a polypeptide according to claim 1.

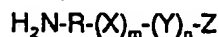
17. A method of enhancing insulin action in a mammal according to claim 16 which comprises iontophoretic transdermal administration.

18. A method of treating Type II diabetes in a mammal which comprises the method according to claim 16.

30 19. A method of enhancing insulin action in a mammal which comprises administering to said mammal an effective amount of a derivative of a polypeptide according to claim 2.

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20. A method of enhancing insulin action in a mammal according to claim 19 which comprises iontophoretic transdermal administration.
21. A method of treating Type II diabetes in a mammal which comprises the method according to claim 19.
- 5 22. A method of enhancing insulin action in a mammal which comprises administering to said mammal an effective amount of a derivative of a polypeptide according to claim 3.
23. A method of enhancing insulin action in a mammal according to claim 22 which comprises iontophoretic transdermal administration.
- 10 24. A method of treating Type II diabetes in a mammal which comprises the method according to claim 22.
25. A method of enhancing insulin action in a mammal which comprises administering to said mammal an effective amount of a derivative of a polypeptide according to claim 4.
- 15 26. A method of enhancing insulin action in a mammal according to claim 25 which comprises iontophoretic transdermal administration.
27. A method of treating Type II diabetes in a mammal which comprises the method according to claim 25.
28. A method of enhancing insulin action in a mammal which comprises
20 iontophoretically administering to said mammal an effective amount of a derivative of a polypeptide comprising the primary structure



- 25 wherein R is an amino acid sequence selected from the group consisting of
His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2),
His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3),
30 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly, and (SEQUENCE ID NO: 4).
His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5);

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and the pharmaceutically-acceptable salts thereof wherein m is zero or one; n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO_2R^1 or CONR^2R^3 wherein R^1 is H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when

- 5 (a) m is one, n is zero and X is a basic L-amino acid residue, or
 (b) m is zero, n is one and Y is a basic L-amino acid residue, or
 (c) m and n are both one and one or both of X and Y are a basic L-amino acid residue;

R^1 is $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when

- 10 (a) m and n are both zero, or
 (b) m is one, n is zero and X is a neutral L-amino acid residue, or
 (c) m is zero, n is one and Y is a neutral L-amino acid residue, or
 (d) m and n are both one and both X and Y are a neutral L-amino acid residue;

- 15 and R^2 and R^3 are each, independently, H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl.

29. A method of enhancing insulin action in a mammal according to claim 28 wherein R is

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2);

- 20 m is zero and n is zero.

30. A method of enhancing insulin action in a mammal according to claim 29 wherein Z is CONR^2R^3 and R^2 and R^3 are each hydrogen.

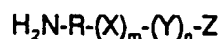
31. A method of treating Type II diabetes in a mammal which comprises the method according to claim 28.

- 25 32. A method of treating Type II diabetes in a mammal which comprises the method according to claim 29.

33. A method of treating Type II diabetes in a mammal which comprises the method according to claim 30.

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34. Use of a derivative of a polypeptide, or a pharmaceutically acceptable salt thereof, comprising the primary structure



for the preparation of a medicament for enhancing insulin action in a mammal by
5 iontophoretic administration thereof, wherein R is an amino acid sequence selected from the group consisting of

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2),

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
10 Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3),

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly, and (SEQUENCE ID NO: 4).

His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5);

15 m is zero or one; n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO_2R^1 or CONR^2R^3 wherein R^1 is H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when

- (a) m is one, n is zero and X is a basic L-amino acid residue, or
- (b) m is zero, n is one and Y is a basic L-amino acid residue, or
- 20 (c) m and n are both one and one or both of X and Y are a basic L-amino acid residue;

R^1 is $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when

- (a) m and n are both zero, or
- (b) m is one, n is zero and X is a neutral L-amino acid residue, or
- 25 (c) m is zero, n is one and Y is a neutral L-amino acid residue, or
- (d) m and n are both one and both X and Y are a neutral L-amino acid residue;

and R^2 and R^3 are each, independently, H or $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl.

35. The use according to claim 34 wherein R is

30 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2);

m is zero and n is zero.

-25-

36. The use according to claim 35 where in Z is CONR^2R^3 and R^2 and R^3 are each hydrogen.

37. A process for preparing a derivative of a polypeptide comprising the primary structure

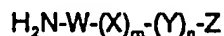
5 $\text{H}_2\text{N-W-COOH}$

wherein W is an amino acid sequence selected from the group consisting of
His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-
Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-
Arg-Gly (SEQUENCE ID NO: 1) and

10 His-Asp-Glu-Phe-Glu-Arg-His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-
Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-
Arg (SEQUENCE ID NO: 6);

and the pharmaceutically-acceptable salts thereof wherein the derivative has a pI of about 4.0 or less, or a pI of about 7.0 or greater and which derivative when processed
15 in a mammal results in a polypeptide derivative having an insulintropic activity, which process comprises preparing a derivative of said primary structure by methods known per se and, optionally, converting said derivative to a pharmaceutically-acceptable salt thereof by methods known per se.

20 38. A process for preparing a derivative of a polypeptide according to claim 37 comprising the primary structure



and the pharmaceutically-acceptable salts thereof wherein m is zero or one; n is zero or one; X is a basic or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is CO_2R^1 or CONR^2R^3 wherein R^1 is H or $(\text{C}_1\text{-C}_6)$ straight or
25 branched-chain alkyl when

- (a) m is one, n is zero and X is a basic L-amino acid residue, or
- (b) m is zero, n is one and Y is a basic L-amino acid residue, or
- (c) m and n are both one and one or both of X and Y are a basic L-amino acid residue;

30 R^1 is $(\text{C}_1\text{-C}_6)$ straight or branched-chain alkyl when

- (a) m and n are both zero, or
- (b) m is one, n is zero and X is a neutral L-amino acid residue, or
- (c) m is zero, n is one and Y is a neutral L-amino acid residue, or

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(d) m and n are both one and both X and Y are a neutral L-amino acid residue;

and R² and R³ are each, independently, H or (C₁-C₆)straight or branched-chain alkyl, which process comprises preparing a derivative of said primary structure by methods known per se and, optionally, converting said derivative to a pharmaceutically-acceptable salt thereof by methods known per se.

39. A process for preparing a derivative of a polypeptide comprising the primary structure



10 wherein R is an amino acid sequence selected from the group consisting of
 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
 Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2),
 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
 Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg (SEQUENCE ID NO: 3),
 15 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-
 Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly (SEQUENCE ID NO: 4) and
 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-
 Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys (SEQUENCE ID NO: 5);

and the pharmaceutically-acceptable salts thereof wherein the derivative has a pI of
 20 about 4.0 or less, or a pI of about 7.0 or greater and having insulinotropic activity,
 provided that said derivative is not a C-terminal (C₁-C₆)straight or branched-chain alkyl
 ester and provided further that said derivative is not a C-terminal carboxamide of the
 formula CONR²R³ wherein R² and R³ are each, independently, H or (C₁-C₆)straight or
 branched-chain alkyl, which process comprises preparing a derivative of said primary
 25 structure by methods known per se and, optionally, converting said derivative to a
 pharmaceutically-acceptable salt thereof by methods known per se.

40. A process for preparing a derivative of a polypeptide according to claim
 39 comprising the primary structure



30 and the pharmaceutically-acceptable salts thereof wherein n is zero or one; X is a basic
 or neutral L-amino acid residue; Y is a basic or neutral L-amino acid residue; and Z is
 CO₂R¹ or CONR²R³ wherein R¹ is H or (C₁-C₆) straight or branched-chain alkyl when

(a) n is zero and X is a basic L-amino acid residue, or

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- (b) n is one and one or both of X and Y are a basic L-amino acid residue; R¹ is (C₁-C₆)straight or branched-chain alkyl when
- (a) n is zero and X is a neutral L-amino acid residue, or
- (b) n is one and both X and Y are a neutral L-amino acid residue;
- 5 and R² and R³ are each, independently, H or (C₁-C₆)straight or branched-chain alkyl, which process comprises preparing a derivative of said primary structure by methods known per se and, optionally, converting said derivative to a pharmaceutically-acceptable salt thereof by methods known per se.
41. A process according to claim 40 wherein R is
- 10 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly (SEQUENCE ID NO: 2).
42. A process according to claim 41 wherein n is zero and X is Arg.
43. A process according to claim 42 wherein Z is CO₂R¹ and R¹ is H, or Z is CONR²R³ and R² and R³ are each H.
- 15 44. A process according to claim 41 wherein n is one and X and Y are each Arg.
45. A process according to claim 44 wherein Z is CO₂R¹ and R¹ is H, or Z is CONR²R³ and R² and R³ are each H.
46. A process according to any one of claims 37-45 wherein said derivative
- 20 is formed by one or more of the following:
- (a) reacting the primary structure with a (C₁-C₆) alkanol in the presence of a catalytic acid to produce a C-terminal (C₁-C₆) alkyl ester thereof;
- (b) reacting the primary structure with a (C₁-C₆) alkanol in the presence of a catalytic acid to produce a (C₁-C₆) alkyl ester of the Asp and/or Glu residues thereof;
- 25 (c) synthesizing a carboxamide derivative of the primary structure by solid phase peptide synthesis;
- (d) deamidating the gln residue thereof;
- (e) alkylating or amidating the free amino group at the N-terminus and/or at the epsilon group of one or more Lys residues of the primary structure, or a
- 30 combination thereof;
- (f) reacting the primary structure with a cyclic anhydride;
- (g) replacing a basic amino acid residue of the primary structure with an acidic or neutral amino acid residue; and
- (h) replacing a neutral amino acid residue with an acidic amino acid residue.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 93/03388

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁴ According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁵ : C 07 K 7/34, A 61 K 37/43														
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 25%; border-bottom: 1px solid black; padding: 5px;">Classification System</td> <td style="border-bottom: 1px solid black; padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px;">IPC⁵</td> <td style="padding: 5px;">C 07 K 7/00, A 61 K 37/00, C 12 N 15/00</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	IPC ⁵	C 07 K 7/00, A 61 K 37/00, C 12 N 15/00								
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IPC ⁵	C 07 K 7/00, A 61 K 37/00, C 12 N 15/00													
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category ⁹</th> <th style="width: 60%; padding: 5px;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 30%; padding: 5px;">Relevant to Claim No. ¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">WO, A1, 91/11 457 (BUCKLEY, DOUGLAS J. et al.) 08 August 1991 (08.08.91), claims 1-7, 12.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-15</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">P, A</td> <td style="padding: 5px;">EP, A2, 0 499 990 (TAKEDA CHEMICAL INDUSTRIES, LTD.) 26 August 1992 (26.08.92), claims 9, 10.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-11</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">Chemical Abstracts, vol. 11, no. 23, issued 1989, December 04 (Columbus, Ohio, USA), M. SUZUKI et al. "Synthesis of GLP-1 related peptides and production of GLP-1 specific antisera", page 634, column 2, the abstract-no. 214 921k, Pept. Chem. 1988, (Pub. 1989), 26th, 73-8.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-15</td> </tr> </tbody> </table>			Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	A	WO, A1, 91/11 457 (BUCKLEY, DOUGLAS J. et al.) 08 August 1991 (08.08.91), claims 1-7, 12.	1-15	P, A	EP, A2, 0 499 990 (TAKEDA CHEMICAL INDUSTRIES, LTD.) 26 August 1992 (26.08.92), claims 9, 10.	1-11	A	Chemical Abstracts, vol. 11, no. 23, issued 1989, December 04 (Columbus, Ohio, USA), M. SUZUKI et al. "Synthesis of GLP-1 related peptides and production of GLP-1 specific antisera", page 634, column 2, the abstract-no. 214 921k, Pept. Chem. 1988, (Pub. 1989), 26th, 73-8.	1-15
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p> </div> </div>														
IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="text-align: center; padding: 5px;">23 July 1993</td> <td style="text-align: center; padding: 5px;">19-08-1993</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">International Searching Authority</td> <td style="border-bottom: 1px solid black; padding: 5px;">Signature of Authorized Officer</td> </tr> <tr> <td style="text-align: center; padding: 5px;">EUROPEAN PATENT OFFICE</td> <td style="text-align: center; padding: 5px;">SCHARF_e.h.</td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	23 July 1993	19-08-1993	International Searching Authority	Signature of Authorized Officer	EUROPEAN PATENT OFFICE	SCHARF_e.h.				
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International Searching Authority	Signature of Authorized Officer													
EUROPEAN PATENT OFFICE	SCHARF_e.h.													

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/03388

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 16-33
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 16-33 are considered to be methods for treatment of the human or animal body by therapy and are subject matter which the International Searching Authority is not required to search under Article 12(2)(a)i) and Rule 39(iv).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/US 93/03388 SAE 73042

In diesem Anhang sind die Mitglieder
der Patentfamilien der im obenge-
nannten internationalen Recherchenbericht
angeführten Patentdokumente angegeben.
Diese Angaben dienen nur zur Unter-
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family
members relating to the patent documents
cited in the above-mentioned inter-
national search report. The Office is
in no way liable for these particulars
which are given merely for the purpose
of information.

La présente annexe indique les
membres de la famille de brevets
relatifs aux documents de brevets cités
dans le rapport de recherche inter-
national visée ci-dessus. Les renseigne-
ments fournis sont donnés à titre indica-
tif et n'engagent pas la responsabilité
de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
WO A1 9111457	08-08-91	CA AA 2073856 EP A1 512042 EP A4 512042	25-07-91 11-11-92 17-02-93
EP A2 499990	26-08-92	CA AA 2061382 EP A3 499990	20-08-92 17-03-93